DEVICE AND METHOD FOR MICROCONTACT PRINTING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Application No. 60/528,993 filed December 12, 2003 the contents of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to microcontact printing devices and a method of fabricating the microcontact printing pins used in such devices.

BACKGROUND

[0003] Microcontact printing of microarrays of many types of biological samples is a popular application for microcontact printing technology. In recent years, the use of silicon-based printing pins has allowed the technology to achieve printing of microarrays having finer sample spot size with improved consistency compared to the stainless steel microspotting pins. But, as the use of the microcontact printing in printing microarrays of biological samples, such as DNA microarrays, continue to grow and new applications for the microcontact printing technology emerges, there is a continual need for improved microcontact printing pins and methods for fabricating such pins.

SUMMARY OF INVENTION

[0004] According to an embodiment of the invention, a pin for depositing a liquid on a substrate is disclosed. The pin comprises a printing tip at a first end and a reservoir, which holds a supply of a printing fluid, communicating with the printing tip. A fluid delivery channel extends between the reservoir and the printing tip for delivering the printing liquid from the reservoir to the printing tip. The channel has a tapered shape decreasing in width from the reservoir to the printing tip. This tapered shape ensures that the delivery of the printing fluid to the printing tip is possible and further more, smooth and consistent. The tapered shape of the channel also allows all of the printing fluid held in the reservoir and the channel to be used up. The printing pin may also have a head portion at its second end that is wider than the rest of the printing pin to provide an area where the pins may be grasped for handling purposes and to prevent the pin from falling through a collimator.

[0005] According to another embodiment of the invention, a pin for depositing a liquid on a substrate includes a printing tip at a first end and a reservoir, which holds a supply of a printing fluid, communicating with the printing tip. The printing pin has a thinned printing tip portion and an non-thinned remainder portion which includes the reservoir, that is thicker than the thinned portion and a stepped portion between the printing tip and the reservoir formed by the change in the thickness between the thinned printing tip portion and the non-thinned remainder portion. A fluid delivery channel extends from the reservoir to the printing tip for delivering the liquid from the reservoir to the printing tip. The stepped portion may be curved. The curve may be formed in a variety of shapes, such as an ellipse or a semi-circle. The stepped portion also helps eliminate

prespotting phenomena by providing wetting force vectors that oppose the gravitational pull on any excess printing fluid on the outer surface of the printing tip and sheeting down towards the printing tip.

[0006] According to another embodiment, a microcontact printing pin holder for use in producing a microarray is disclosed. The pin holder comprises a first planar member, a first aperture extending through the planar member for receiving a pin that deposits a predetermined volume of a liquid on a substrate to produce the microarray, and an elastomeric member provided at a distance above the first planar member.

[0007] The pin holder may also include a second planar member having a second aperture extending therethrough for receiving a bottom portion of the pin. The second planar member is disposed under the first planar member such that the apertures are in axial alignment with one another. The pins and the pin holder of the invention described herein may be microfabricated from a material selected from the group consisting of semiconductors, polymers, ceramics, and non-ferric alloys.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] All drawings are schematic and are not to scale. Like reference numerals used in the drawings refer to like structures.

[0009] FIGS. 1A-1G illustrate the microfabrication of microcontact printing pins and pin holders according to an exemplary embodiment of the invention.

[0010] FIG. 2 is a plan view of a microcontact printing pin of the invention fabricated with the microfabrication process illustrated in FIGS. 1A-1G.

[0011] FIGS. 3A-3D illustrate a variety of printing pin thinning processes according to an embodiment of the invention.

[0012] FIGS. 3E and 3F illustrate the printing pin layouts in relation to wet etched pits on a silicon wafer stock being processed according to an embodiment of the microfabrication process of the invention.

[0013] FIG. 4 is an isometric view of the printing tip end of a microcontact printing pin that has been thinned by a microfabrication process according to an embodiment of the invention.

[0014] FIGS. 5A-5I illustrate microfabrication process steps for forming an embodiment of a printing pin having a thinned printing tip according to another embodiment of the invention.

[0015] FIGS. 6A-6E illustrate microfabrication process steps for forming another embodiment of a printing pin having a thinned printing tip according to another embodiment of the invention.

[0016] FIG. 6F is a plan view of a microcontact printing pin whose printing tip end has been thinned by the microfabrication process illustrated in FIGS. 6A-6D.

[0017] FIG. 7A is a perspective view of the printing tip end of a microcontact printing pin according to an embodiment of the invention holding an amount of printing fluid after a fluid pick up.

[0018] FIGS. 7B and 7C are side elevational views of the microcontact printing pin of FIG. 7A, printing a print spot on a substrate.

[0019] FIG. 7D is a perspective view of another embodiment of the printing tip end of the microcontact printing pin of FIG. 7A.

[0020] FIG. 8A is a perspective view of the printing tip end of the microcontact printing pin of FIG. 6E holding an amount of printing fluid after a fluid pick up.

[0021] FIGS. 8B-8C are side elevational views of the microcontact printing pin of FIG. 8A, printing a print spot on a substrate.

[0022] FIG. 8D is a side elevational view of the microcontact printing pin of FIG. 6E after some of the printing fluid has been depleted.

[0023] FIG. 8E is a plot comparing the print spot size profile between a microcontact printing pin of FIG. 7A and the microcontact printing pin of FIG. 8A.

[0024] FIG. 9 is a perspective view illustration of a microcontact printing pin according to an embodiment of the invention.

[0025] FIGS. 10A-10C are isometric views of a variety of printing tip configurations according to an embodiment of the invention.

[0026] FIG. 11A is a plan view of a section of a pin holder according to an exemplary embodiment of the invention.

[0027] FIG. 11B is an elevational view of the pin holder.

[0028] FIG. 12A is an elevational view of the pin holder according to another embodiment of the invention.

[0029] FIG. 12B are elevational views illustrating the operational advantage of the pin holder of FIG. 12A.

[0030] FIGS. 13A and 13B are elevational views illustrating the operation of the pin holders of FIGS. 12B-A and 12B-C.

[0031] FIGS. 14A and 14B are elevational views of another embodiment of the pin holders of FIGS. 13A and 13B.

DETAILED DESCRIPTION OF THE INVENTION

[0032] FIGS. 1A-1G illustrate the microfabrication of silicon-based pins **20**, having non-thinned printing tip, and pin holders (see the planar member **141** of FIG. 11A) according to an exemplary embodiment of the present invention using conventional silicon microfabrication methods. First, pin and pin holder design data is used to design a photo mask **86** (see FIG. 1E). The design of the photo mask **86** may be prepared using any suitable CAD software program, such as AutoCAD®. The photo mask **86** may then be prepared, for example, by generating a negative image of the design in chromium on a long wavelength UV transparent glass substrate.

[0033] As shown in FIGS. 1A and 1B, a first layer of photoresist **82** may be deposited onto a first silicon wafer **80**. The first silicon wafer **80** may be made from single crystal silicon having a (100) crystal orientation, with both sides polished and about **200** μm thick. The first layer of photoresist **82** may be deposited, for example, using a conventional spin coating technique.

[0034] In FIG. 1C, a second silicon wafer **84** (component wafer **84**) is bonded on top of the first silicon wafer **80** (support wafer **80**) by placing the second wafer **84** on top of the first layer of photoresist **82** and soft-baking the first layer of photoresist **82** for about 1 and 2 minutes at approximately 90°. The second silicon wafer **80** may also be made from single crystal silicon having a (100) crystal orientation, with both sides polished and about 200 μm thick. The first layer of photoresist **82** between the wafers **80, 84** prevents severe undercutting of the component wafer **84** when etchant travels therethrough. Such an etchant is used when, for example, Reactive Ion Etching (RIE) micromachining is used. One of ordinary skill in the microfabrication art will of course recognize that any

other suitable bonding material or method may be used to bond the two wafers **80**, **84** together.

[0035] As shown in FIG. 1D, a second layer of photoresist **85** is deposited over the component wafer **84** and soft-baked. The second layer of photoresist **85** layer is patterned as shown in FIG. 1E, by placing the photo mask over the second layer of photoresist **85**, irradiating the wafers **80**, **84** and developing the second layer of photoresist **85**. The irradiated portions **87** of the second layer of the photoresist **85** are removed from the component wafer **84**, thus, leaving a photoresist pattern thereon, which is made up of the non-irradiated regions of photoresist **88**.

[0036] In FIG. 1F, the pins and holders are micromachined from the component wafer **84** using any conventional silicon micromachining technique, such as Deep Reactive Ion Etching (DRIE). As is well known in the silicon microfabrication art, the micromachining process removes the portions of the silicon wafer not protected by the photoresist. Dry etching techniques such as plasma etching are used for etching features with variable tapering and high aspect ratio microstructures. The most common forms of dry etching for micromaching or microfabrication applications are isotropic ion etching and anisotropic DRIE. Unlike anisotropic wet etching, DRIE etching is not controlled by the relative etch rates of the silicon crystal planes and, thus, deep channels and pits up to few tens of microns deep with nearly vertical walls and of arbitrary shape can be etched using anisotropic DRIE technique.

[0037] The general layout of the pins **20** on a section of the component wafer **84** is shown in FIG. 1G. As can be seen mounting heads **26** of the pins **20** may be packed closely together with the shafts **22** filling most of the space when the pins **20** are formed

in an interdigitated pattern. This efficient space filling allows the maximum number of pins to be fabricated per unit area of wafer surface.

[0038] The component wafer **84** is machined all the way through as shown in FIG. 1F to separate the pins and pin holders. The separated pin and pin holders are removed from the support wafer **80** by dissolving the first and layer of photoresist **82** with solvent (the solvent also removes the patterned sections **88** of the second layer of photoresist **85** from the components). After several thorough washings in fresh solvent, the separated pins **20** and pin holder components are oxidized using conventional well known silicon oxidizing methods to form a coating of (typically about 0.5 to 1 μm thick) SiO_2 hydrophilic film layer on the components. At this stage, the pins **20** and the pin holder may be assembled.

[0039] Referring to FIGS. 1G and 2, the microcontact printing pins **20** comprise shaft **22** having a head portion **26** at one end thereof and a printing end at the opposite end. The printing end comprises a reservoir **14** for holding a supply of printing fluid, a printing tip **15**, and a delivery channel **12** in communication with the reservoir **14** and the printing tip **15**. The channel **12** delivers the fluid from the reservoir **14** to the printing tip **15**. The channel **12** divides the printing tip **15** into two prongs and the ends of each prong are printing end wall surfaces **17**. The printing end wall surfaces **17** maybe substantially flat but preferably textured or contoured for optimized printing. This aspect of the invention will be further discussed below.

[0040] The smoothness (rms roughness) of the DRIE cut surfaces are typically well below 1 μm and 5 μm features are easy to fabricate. Most of the exposed surface of the pin, which corresponds to the polished surfaces of the wafer covered by photoresist during the DRIE treatment, has a roughness only in the tens of Angstroms. This

smoothness abrogates the need for the shaft-polishing step required for the steel, which is necessary for the shaft to slide freely in its holder. Since the holder for the silicon pins is also microfabricated, the high tolerances and smooth surfaces allow for a high precision, but smooth fit during the movement of the pin in the holder during printing. Accordingly, the pins and holders have a very smooth, mirror like finish and slide without restriction. Although the machining accuracy of each pin is important, it is also imperative that the uniformity of all pins manufactured is accordingly as high. Batch-to-batch uniformity is one of the great strengths of silicon microfabrication and typically all the components are essentially identical yielding more uniform microarrays. The fabrication of complex pin shapes and the cutting of intricate features into the pins are simple with this fabrication technique, limited only by the achievable feature size, limitations of the cutting technique and the mechanical strength of the part.

[0041] The pins and pin holders may be assembled together by placing a desired number of the pins into each of the pin holders. This may be accomplished with the aid of a vacuum tweezers, which grasps the mounting head of the pin. Each pin is dropped into a desired slot in the pin holder with the aid of a small plastic funnel that guides the pin into the slot.

[0042] According to the microfabrication process described above, the microcontact printing pins **20** microfabricated out of silicon wafers retain the thickness of the particular silicon wafer **84** used, typically about 200 μm. To produce sample print spot sizes that are smaller than 200 μm, and particularly print spot sizes of 100 μm or smaller diameter, printing pins having printing tips having dimensions that are substantially smaller than the thickness of the stock silicon wafers is necessary.

[0043] In the microfabrication process described in reference to FIGS. 1A-1G according to an embodiment of the invention, because the pins **20** are cut from the wafer **84** using an anisotropic plasma etch (DRIE), which cuts perpendicular trenches to the wafer surface, and the plane of the cut lies in the plane of the wafer **84** during fabrication, one of the printing tip **15** dimensions has to correspond to the wafer thickness. Thus, in order to fabricate printing pins having printing tips that are smaller than 200 μm, thinner silicon wafer stocks are needed. However, it is not practical to make thinner silicon wafers for practical handling reasons. For example, 100 μm thick wafers are very fragile and difficult to handle and, thus, it would not be practically feasible to use 100 μm thick silicon wafers to microfabricate print pins having printing tips of 100 μm width.

[0044] One way of resolving this problem is to selectively thin the printing pin's printing tip region to shape the printing tip to any desired dimension smaller than the thickness of the starting silicon wafer. The thinning process according to an embodiment of the invention uses either a combination of wet KOH and DRIE etching, or DRIE etching alone, to sculpt the printing tip to the desired shape and dimension by selective thinning process before the pins are cut from the stock wafer.

[0045] Referring to FIGS. 3A-3D, four basic printing tip shapes may be fabricated depending on whether a wet or DRIE etch is used for the thinning operation which will provide depressions with sloped or vertical sidewalls in a wafer **200**, usually a (100) oriented silicon wafer. One example of wet etched design choice is shown in FIGS. 3A and 3B. Unlike DRIE etching, the wet etch process can be run in parallel. By using either a double or single sided etching procedure, the printing pin tip is shaped

symmetrically or asymmetrically, respectively, between the two large faces of the starting silicon wafer **200**.

[0046] FIG. 3A is a sectional view of a silicon wafer **200** in which pits **202** are formed on both faces of the silicon wafer **200** using wet KOH etching process. As shown, because wet KOH etches pits with the bottom of the pit formed from (100) crystallographic plane of the silicon wafer **200** and the sides from (111) crystallographic planes, the pits **202** have sloped sides. FIG. 3B is a sectional view of a silicon wafer **200** in which a pit **204** is formed on one face of the silicon wafer **200** using wet KOH etching process. In the embodiments shown in FIGS. 3C and 3D, the pits **206, 208** are formed with DRIE etching process. Unlike the anisotropic wet KOH etching , DRIE etching is not controlled by the relative etch rates of the silicon crystal planes and, thus, the pits **206** and **208** have vertical sides. In examples illustrated in FIGS. 3A-3D, by cutting the wafer **200** through the broken line of the etched pits **202, 204, 206**, and **208** produces two identical printing tips, one of which is shown as **210, 212, 214**, and **216** for each of the cuts.

[0047] FIGS. 3E and 3F illustrate plan view schematic layout showing the outlines of the printing pins **210** and **212,** from FIGS. 3A and 3B, overlaid with the thinning pits **202** and **204**. The thinning pit **204** in this view is shown in broken lines to illustrate that it is only on the far side of the silicon wafer **200** being viewed. The printing pins **210** of FIG. 3E are thinned symmetrically from both sides of the wafer **200**. The printing pins **212** of FIG. 3F are thinned asymmetrically from one side of the wafer **200**.

[0048] Next, the outline pattern of the printing pins **210** and **212** is cut by the DRIE etching. For the printing pins **210** which have sloped sidewalls formed by the thinning

pits **202** on both sides of the wafer **200**, the use of projection lithography is required to pattern the pit surface with the outline of the printing pins **210** before they can be cut by the DRIE etching process. In the case of the printing pins **212**, which have the thinning pit **204** only on one side of the wafer **200**, the DRIE etching cut may be conducted from the flat side of the wafer **200**. Then, the outline pattern of the printing pins **212** may be transferred to the flat surface of the wafer **200** using routine photolithography.

[0049] Referring to FIG. 4, one method of reducing the print tip dimensions according to an embodiment of the invention is disclosed. FIG. 4 shows the symmetric printing tip **17** that results when the pins are cut from a substrate that has been thinned on both sides, as shown in FIG. 3E, with a KOH etch. Starting from the original thickness $\mathbf{D_{200}}$ of the wafer **200**, the wet etched pit **202** forms the sloped surfaces **202a** (corresponding to the <111> plane of the silicon wafer) and the horizontal surfaces **202b** symmetrically from both large faces of the wafer **200**. Next, the wafer **200** is cut by DRIE etching in the direction **C**, shown in FIG. 4, transverse to the large faces of the <100> orientation silicon wafer **200** to further reduce the print tip **17** to the final dimensions **x, y**. To reiterate, in this embodiment of the invention, wet KOH etching is used to obtain the **y** dimension of the printing tip **17**. The wet KOH etching thins the <100> orientation silicon wafer **200** in the <100> crystal plane direction creating sloped side walls **202a** which are in the <111> crystal plane of the silicon wafer **200**. Then, the **x** dimension of the printing tip **17**, and the entire outline of the pin, is obtained by cutting the wafer **200** in the direction **C** by DRIE etching, the direction **C** being orthogonal to the <100> crystal plane. The DRIE etching is used to cut through the resulting structure to form the fluid reservoir **14** and the fluid delivery channel **12**.

[0050] Referring to FIGS. 5A-5H, another embodiment of printing tip thinning process will be described in which the square shaped printing end wall surface **17** of FIG. 4 may be further processed into an octagonal shaped tip. Such an octagonal shape may be more desirable in certain applications because the octagonal shape better approximates a circular printing tip. As shown in FIG. 5A-5D, a <100> oriented silicon wafer **200** is wet etched from both sides of the wafer **200** in a thinning operation to thin a portion of the wafer **200** down to the ultimate horizontal thickness **hh** of the resulting printing end wall surface **17** (see FIGS. 5G and 5H). To do this, first, the portions of the two faces of the wafer **200** that is not to be thinned, identified as region(s) **230a** in FIG. 5A, are protected by forming a coating of etch stop material. The wet etch step usually uses KOH etchant, as discussed above, and for KOH, SiO_2 will suffice as etch stop for typical etch durations involved in this etch depth (less than 100 μm). If more etch stop protection is required, a coating of Si_3N_4 may be used. This is achieved by oxidizing the wafer surfaces by oxidizing in steam at >900°C to form a dense, thick (0.5-1.0 μm) coating of SiO_2. The SiO_2 coated wafer surface is then patterned by photolithography and selective removal of the SiO_2 coating from the region to be thinned **230b**. FIG. 5B shows an elevational view of the side face **220** of the wafer **200** which will eventually form the printing end tip surface **17**. Next, the region **230b** is thinned by wet KOH etch for an appropriate duration until the side face **220** reaches the thickness **hh**. FIGS. 5C and 5D show the resulting structure. As discussed above in reference to FIGS. 3A, 3B, and 4, this wet KOH etching produces sloped side walls **234** which are the <111> crystal planes of the silicon wafer **200**. The horizontal surface **232** of the thinned portion is the surface parallel to the <100> crystal plane. FIG. 5D shows an elevational view of the side face

220 which is now thinned to the thickness **hh**. Next, the regions **232a** (in the <100> plane) and the sloped side walls **234** are coated with the SiO_2 etch stop layer using photolithography process as described above. The surfaces marked **232a** will eventually form two of the eight sides of the octagonal shaped printing end wall surface **17**. Next, another wet KOH etching step is carried out, further thinning the portions of surface **232** which are not protected by the etch stop layer (the region 232a). FIGS. 5E and 5F illustrate the resulting structure. The etch stop protected regions **230a**, **234**, and **232a** remain as before but the unprotected regions of the surface **232** has been further thinned down to surface **236**. The portion of the side face **220** between the protected surfaces **232a** now show six of the eight sides necessary to form an octagon. The region between the protected surfaces **232a** has retained the thickness **hh**. Next, the structure of FIG. 5E is again patterned with an etch stop layer through photolithography to make the final etching step to form the octagonal printing end wall surface **17**. As in the previous embodiment, the final etch step is conducted by DRIE etching process. The etch stop layer pattern is as shown in the plan view of FIG. 5G. The non-shaded surfaces are protected by etch stop and the shaded areas are to be cut away by DRIE etching. As noted in FIG. 5G, the pattern for the fluid delivery channel **212** (FIG. 5H) may be created at this time so that the final shaping of the printing end wall surface **17** and the channel **212** can be formed with one DRIE etching step. The etch stop material may be SiO_2 or a photoresist. The resulting octagonal shaped printing end wall surface **17** can be seen in the printing pin tip structure shown in FIG. 5H and 5I. The DRIE etching has cutaway the shaded areas in FIG. 5G forming the vertical surfaces **238** creating the octagonal shaped printing tip. The fluid delivery channel **212** is also formed.

[0051] Referring to FIGS. 6A-6E, another method of thinning the printing pin tip according to an embodiment of the invention is disclosed. This method relies on the use of DRIE for all of the etching steps. As shown in FIG. 6A, the <100> orientated silicon wafer **200** is patterned with tips mask **301** with a pin pattern **P1** on the first side **262**. The pin pattern **P1** includes the entire outline of the printing pin, the fluid delivery channel and reservoir (not shown),. Next, the first side **262** of the wafer **200** is etched by DRIE etching until a desired thickness **D2** is removed. The thickness **D2**, for example, may be about 100 μm. FIG. 6B shows the removed silicon wafer **200** material in broken lines. The structure **PP1** left behind is half of the printing. The wafer **200** is then flipped upside down (FIG. 6C) and the second side **264** of the wafer **200** is patterned with a thinning pattern **P2** using a second tips mask **302**. The thinning pattern **P2** includes the outline of the portion of printing pin tip that is to be removed. The wafer **200** is then DRIE etched second time from the second side **264** of the wafer **200** to remove all of the remaining wafer **200**, represented by the broken lines in FIG. 6D, leaving behind the structures **PP2** and **PP1** which form one contiguous part, the printing pin **30**. The thickness of the thinned printing tip **15** is **D2**, defined by the structure **PP1**. The unthinned portion of the printing pin **30** retains the thickness **D1** of the silicon wafer **200**. Because of this change in thickness between the thinned printing tip **15** and the unthinned remainder portion of the printing pin **30**, a stepped portion **18** is created. As shown in FIG. 6E, the stepped portion **18** comprises a surface that is substantially orthogonal to the longitudinal axis **L** (FIG. 6F) of the pin **30**. FIG. 6F is a plan view illustration of the printing pin **30** showing its full outline. The printing pin **30** comprises an elongated shaft **22**, a head portion **26** at one end and the printing end at the opposite end of the shaft **22**. The printing end

includes the thinned printing tip **15**, a fluid reservoir **14** provided apart from the printing tip **15** for holding a supply of printing fluid. The printing end also includes a fluid delivery channel **12** extending between the reservoir **14** and the printing tip **15**. The stepped portion **18** is provided between the printing tip and the reservoir **14**. As will be further described below, the stepped portion **18** serves to eliminate an undesirable prespotting phenomena.

[0052] Referring to FIG. 6F, microcontact printing pin **30** is a printing pin microfabricated by the all-DRIE process of the invention described above. The stepped portion **18** between the reservoir **14** and the printing tip **15** formed by the difference in the thickness between the thinned and unthinned portions of the printing pin **30** provides another benefit of directing any printing fluid on the outer surface of the pin into the dispensing channel **12** and to the printing tip **15**, thus, eliminating prespotting phenomenon with certain printing tips. As illustrated in FIG. 7A, in a microcontact printing pin **20** that does not have a thinned printing tip and, thus, does not have a stepped portion, when the microcontact printing pin **20** is dipped in the printing fluid for fluid pickup, some excess fluid **55** wets and adheres to the outer walls **50** of the pin **20**. FIG. 7B is a side elevational view of the microcontact printing pin **20** after a printing fluid pickup having some printing fluid **55** adhering to the outer surface **50** of the pin **20** and positioned over a substrate **S**. As shown in FIG. 7C, when the printing pin **20** touches down on the substrate **S**, the fluid **55** that was adhering to the outer surface **50** of the printing pin **20** wets to the substrate **S** and dispenses additional amount of the printing fluid **56** on to the substrate S, resulting in a print spot that is larger than intended. This phenomena is referred to herein as prespotting. For a solution like water or aqueous

solutions of DNA or proteins in contact with a wettable surface, like the SiO_2 surface of the pin, there is an attractive force perpendicular to the surface holding the liquid to the surface which can be represented as a vector pointing perpendicular to the surface. In certain pin tip shapes such as those shown in Figs. 8B and 8C, because the surface of the stepped portion **18** is substantially orthogonal to the longitudinal axis L (FIG. 6F) of the pin **30**, the wetting force vector **V** is 180° away from the direction of the print fluid sheeting down the external pin shaft surface toward the substrate and therefore prevents said fluid on the external shaft from reaching the substrate thereby preventing the prespotting phenomena. Thus, the surface of the stepped portion 18 being orthogonal to the longitudinal axis L (which is vertical and parallel to the direction of the gravitational pull while the printing pins **30** are in operation) provides the optimal orientation for the wetting force vector **V**, *i.e.*, directly opposing the gravitational pull on the print fluid sheeting down the external pin shaft surface. The prespotting phenomena leads to highly variable and oversized spots thereby introducing difficulties into the intensity analysis, increasing the difficulties in spot to spot comparisons and decreasing confidence in results in general.

[0053] The microcontact printing pin **30** of FIG. 6F having the stepped portion **18** after it has picked up some printing fluid (FIG. 8A). Because of the surface reasons given above, the excess fluid **25** wetting the outer surface of the pin **30** tends to collect near the stepped portion **18** away from the printing tip **15**. As illustrated in FIGS. 8B and 8C, when the printing pin **30** touches down on the substrate **S**, only the intended amount of the printing fluid is dispensed from the dispensing channel **12** at the printing tip **15** forming the print spot **27**. The excess fluid **25** is held away from the printing tip **15** and

the substrate **S** by wetting to the stepped portion **18**. As the printing fluid depletes through further printing, the excess fluid **25** is drawn into the dispensing channel **12** and retracts further away from the printing tip **15** as illustrated in FIG. 8D. The consistency and repeatability of the print spot sizes produced by the thinned printing tips on printing pins of the invention is graphically illustrated in FIG. 8E. FIG. 8E shows the spot profile from the first to the last spot printed from a single sample uptake using the thinned printing pin **30** having the stepped portion **18**, as shown in FIGS. 6F and 8A, and the printing pin **20** type shown in FIG. 7A which does not have the stepped portion. The thinned printing pin **30** is able to produce much more consistent print spot sizes.

[0054] According to another embodiment, the non-thinned printing pin **20** of FIG. 7A may be modified with grooves **57** on the external walls **50** to produce the same effect of preventing prespotting phenomena as the stepped portion **18** of the printing pins **30**.

[0055] Referring to FIGS. 6E and 6F, it should be noted that the stepped portion **18** is not a straight ledge but is curved. The curved shape of the stepped portion **18** assists in distributing the stress of the thinned discontinuous structure more widely than a linear cut would. In this exemplary example, the curve approximates a section of an ellipse, however, a variety of other curve shapes, a semi-circle for example, would work.

[0056] Referring to FIGS. 2 and 9, the printing end of a microcontact pin **20** according to an aspect of the invention is disclosed. The printing end of the printing pin **20** has a printing tip **15** and a reservoir **14** for holding a supply of printing fluid provided apart from the printing tip **15**. The structures of the printing tip **15** including but not limited to the reservoir **14** and the channel **12** are configured and dimensioned to optimize the microcontact printing process. The printing tip **15** end of the printing pin **20** is formed

with two side wall surfaces **16** that gradually taper toward the printing tip **15**. The printing tip **15** is separated into two substantially flat printing end wall surfaces **17** oriented generally perpendicular to the center line **CL** of the printing pin **20**, such that the surfaces **17** are generally parallel to the surface of a substrate to be printed.

[0057] The reservoir **14** and the printing tip **15** are connected by an elongated dispensing channel **12** to enable delivery of the printing fluid from the reservoir **14** to the printing tip **15**. The dispensing channel **12** has a larger width **W1** at the reservoir end and a smaller width **W2** at the printing tip **15**. The width of the dispensing channel **12** changes gradually and constantly between the reservoir **14** and the printing tip **15** without any abrupt changes. In other words, the dispensing channel **12** has a tapered shape. This tapered shape of the dispensing channel **12,** in addition to enabling smooth, accurate and controllable delivery of the printing fluid from the reservoir **14** to the printing tip **15,** also very importantly serves to ensure that 100% of the sample taken up into the reservoir **14** and the channel **12** can be delivered to the printing tip **15**. When the channel **12** tapers toward the printing tip **15**, the meniscus at the top of the reservoir shaft retreats toward the print tip **15** as the reservoir fluid is depleted delivering all of the sample to the printing tip **15**. The width **W2** of the dispensing channel **12** at the printing tip **15** may be from about 10 nm to several hundred micrometers depending on the thickness of the printing tip **15**. The length of the channel **12** can be from several nanometers to several centimeters in length with a preferred length of 1 μm to 50 mm. The degree of taper (defined here as channel width **W2** at exit printing tip **15** divided by the width **W1** at top of reservoir) over this length can range from about one to about zero with a preferred range between one and 1/10.

[0058] Generally, in a conventional printing pin whose dispensing channel has a constant width from the reservoir to the printing tip, as the printing fluid depletes through multiple printing steps and the overall volume of the printing fluid held in the dispensing channel and the reservoir decreases, a meniscus will form in the dispensing channel at the printing tip and the printing fluid will be drawn back up the dispensing channel away from the printing tip. Because the printing tip is not sufficiently wet with the dispensing fluid, dispensing will be inconsistent from one printing spot to the next and the dispensing fluid may not even dispense.

[0059] Generally, the printing fluids used with the printing pins of the invention, such as the printing pin **20** are aqueous fluid. And for aqueous printing fluid, the tapered shape of the dispensing channel **12** provides another beneficial function. Because the dispensing channel **12** narrows towards the printing tip **15**, and a narrow channel will withdraw liquid from a larger channel of the same depth as the fluid is depleted, the printing tip **15** remains wet even as the printing fluid is depleted from the reservoir and channel. This provides a constant and smooth delivery of the dispensing fluid to the printing tip **15** and utilizes essentially 100% of the sample taken up. The constant and smooth delivery of the dispensing fluid, in turn, helps maintain a consistent print spot size from one print spot to the next and preferably through a series of print spots until the printing fluid held in the reservoir **14** and the dispensing channel **12** is consumed. This beneficial effect of the tapered dispensing channel **12** occurs in this embodiment because the dispensing fluid is an aqueous fluid having polar molecules which wets well to the printing pin surface **15** made from silicon dioxide. Aqueous fluid wets well to the silicon-based printing pin **15** because silicon material has a thin coating of native oxide

which naturally forms from exposure to the atmosphere. A more conformal, more durable and thicker coating is made from treating the silicon with steam at 900°C in air. Aqueous fluid wets well to the native oxide surface because the native oxide, which is SiO_2 is also a polar material.

[0060] According to another embodiment, because the native oxide on the silicon surface may not be consistent or thick enough, the native oxide layer may be enhanced by forming a thick, dense, and continuous SiO_2 layer. The thick SiO_2, about 0.5 to 1.0 μm thick, may be formed by treating the silicon-based printing pin with steam at 900-1000°C. The thick continuous SiO_2 coating protects the printing pins **15** from certain chemicals and provides a surface that is easily cleaned and regenerated by heating in the atmosphere or under oxygen. These heating treatments are particularly effective at removing any biological or organic impurities.

[0061] Another advantage of the thick SiO_2 coatings on the silicon microcontact printing pins is that from a surface chemistry viewpoint, the SiO_2 surface is identical to glass and thus water or aqueous sample solutions will wet very well to the surface which is necessary for the proper functioning of the printing pins of the invention. Also, by attaching certain chemicals to the surface of the SiO_2, the surface properties of the printing pins may be modified to alter the wetting properties or biological species (*e.g.* proteins, antibodies, or DNA) can be attached to the SiO_2 surface to greatly increase the molecular specificity. For example, various silanes, such as, trimethylchlorosilane may be added to the SiO_2 surface to make a portion of the surface hydrophobic if necessary.

[0062] Referring to FIG. 9, another aspect is the depth of the dispensing channel **D**, which may be 200 μm (the thickness of the stock silicon wafer used to microfabricate the

printing pin), but could also range from 10 nm to several millimeters. The depth **D** greater than 200 μm can be achieved by using wafers thicker than 200 μm.

[0063] Referring to FIGS. 10A-10C, various other configurations for the printing tip **15** of a microcontact printing pin **20** according to another embodiment are disclosed. While some of the current microcontact printing pin's printing tips are flat, *i.e.* substantially parallel to the substrate on which the printing is conducted, the quality of the printing spots can be improved in terms of consistency of the spot size can be improved if the printing tip **15** is fabricated to have non-flat printing end wall surfaces **17**. FIG. 10A illustrates a printing tip **15a** where the printing end wall surfaces **17** have curved surfaces. FIG. 10B illustrates a printing tip **15b** where the printing end wall surfaces **17** have scalloped surfaces. FIG. 10C illustrates a printing tip **15c** where the printing end wall surfaces **17** have sloped surfaces. These examples of non-flat printing end wall surfaces **17** slightly increase the volume of the printing fluid, also referred to as the touch off volume, held at the printing tip by increasing the surface area of the printing end wall surfaces **17** to which the printing fluid wets. The non-flat surface also creates cavity like space(s) at the printing tip **15** which also increases the volume of the printing fluid being held at the printing tip. In the exemplary printing tips **15a, 15b,** and **15b** illustrated in FIGS. 10A-10C, respectively, cavity or cavity-like space(s) **18** defined between the non-flat printing end wall surface **17** and the tangent line **T** represent the slight increase in the touch off volume. The tangent line **T** represents the substrate surface on to which the printing tips **15a, 15b, 15c** would print to. In the embodiment of FIG. 10C, the cavity **18** is defined by sloped faces **17** of the printing tip **15c** that are oriented at an acute angle θ relative to the center line **CL** of the pin. Increasing the volume of the printing fluid at the

printing tip provide a slightly larger touch off volume which improves the shape and volume of the resultant print spot. The larger tip volume may also allow the same amount of printing fluid to be printed with a lighter than normal touch-off pressure.

[0064] The configuration and dimensions of the printing tips on the various embodiments of the printing pins discussed herein according to the invention can be adjusted so that the volume of printing liquid sample deposited by each printing pin and/or the area of the spotted liquid sample (spot) can be varied as desired. It is contemplated that, for example, the configuration and dimensions of the printing tips on the printing pins discussed herein can be adjusted so that the volume of liquid sample deposited by each pin can be as large as about 0.1 milliliters (mL), and as minute as about 10-4 picoliter (pL), or any volume between about 0.1 mL and 10-4 pL. Similarly, the configuration and dimensions of the printing tips can be adjusted so that the area of the liquid sample spots deposited by each pin can be as large as about 10 square millimeters (mm^2), and as minute as about 10^{-6} square microns (μm^2), or any area between about 10 mm^2 and about 10^{-6} μm^2. There are trade-offs among these dimensions that must be balanced. For instance, increasing the dimensions of the major and minor axes of the reservoir to increase the volume thereof in order to decrease the number of fill steps can compromise the mechanical stability of the printing pin's shaft.

[0065] FIGS. 11A and 11B illustrate an exemplary embodiment of the pin holder **140** of the invention. The pin holder **140** is typically configured as a planar member **141** having an array of rectangular, microfabricated slots **142** extending therethrough, each of the slots **142** accepting a microcontact printing pins **120** of the invention. Printing pins **120** may be any one of the embodiments of the printing pins illustrated by the printing pin **30**

of FIG. 6E or the printing pin **20** of FIG. 9. The configuration and dimensions of the pin holder **140** may be varied to accommodate up to 100,000 microcontact printing pins **120** of the invention. In one illustrative embodiment, the pin holder **140** may be 10 cm by 16 cm. The configuration and dimensions of the slots **142** may also be adjusted to provide a pin density, *i.e.*, the number of pins per unit area of the holder, of about 1 pin per 10 mm^2 of holder area to about 10^6 pins per mm^2 of holder area. The pin density of the pin holder **140** is important as it determines the spot density of the microarray of samples, such as DNA samples, printed by the assembly of the pin holder **140** and printing pins **120**. The slots **142** of the pin holder **140** are also configured and dimensioned to allow the shafts **22** of the pins **120** to be slip-fitted into the slots **142** in a frictionless manner with no lateral movement, and suspended by their mounting heads **26**, which rest on the upper surface **144** of the pin holder **140**, while preventing rotation of the pins **120** in the slots **142**.

[0066] FIG. 12A illustrates a second exemplary embodiment of a pin holder **150** of the invention. In this embodiment, upper and lower planar members **152**, **154**, respectively, are bonded together by a perimeter spacer **156** in a single unit referred to herein as a collimating holder **150**. Each of the upper and lower planar members **152** and **154** are structured substantially same as the planar member **141**. The collimating holder **150** is used to prevent the microcontact pins **120** from "tipping over" when touching the substrate **S** as shown in FIG. 12B. More specifically, when the pins **120** touch the substrate **S** during printing, the pins **120** may be excessively raised out of the "non-collimated" holder **140** of the previous embodiment such that the head portions **26** of the printing pins **120** no longer touch the upper surface **144** of the planar member **141** to prevent the pins **120** from tipping over. The collimating holder **150** solves this problem

by providing the lower planar member **154**, which guides the bottom portion of the pin shafts **22** to maintain the vertical orientation of the pins **120** in the collimating holder **150**. The pin holder may be microfabricated from a material selected from the group consisting of semiconductors, polymers, ceramics, and non-ferric alloys.

[0067] In the exemplary embodiment of FIG. 11A, 1536 slots **142** may be provided in the planar member **141** of the pin holder **140** (or in the upper and lower planar members **152**, **154** of the collimating holder **150** of FIG. 12A) and the slots **142** may have a center-to-center spacing $\mathbf{H_{SP}}$ of 2.25 mm. One of ordinary skill in the art will recognize that this embodiment of the pin holder may be advantageously used with a conventional 1536 well microtiter plate (which holds the sample solutions and is not shown herein), as the wells of the microtiter plate have the same 2.25 mm center-to-center spacing as the slots of this exemplary pin holder. Hence, 1536 pins can be installed in the pin holder and dipped directly into all 1536 wells of the microtiter plate, or, with every other pin removed, into a conventional 384 well microtiter plate (which has a 4.5 mm center-to-center well spacing).

[0068] Similar to a fountain pen, the microcontact printing pins **120** produces print spots optimally when the printing pins **120** contact the substrate with a certain amount of contact pressure. The specific contact pressure would depend on the particular dimensions of the printing pins **120**, the type of printing fluid involved and the type and surface characteristics of the substrate. In the pin holder **140** and the collimating holder **150** described above, the contact pressure exerted by the printing pins **120** on the substrate **S** is generated by the weight of the printing pins **120** themselves. These are generally referred to as floating pins. As illustrate in FIGS. 13A and 13B, during the

microarray printing process, the pin holders **140** and **150** are lowered towards the substrate **S** until the top surfaces of the planar members **141** and the **152** of the pin holders **140, 150**, respectively are distance **h** (hereinafter, "drop distance") apart from the head portions **26** of the pins **120**. Thus, the weight of the pins **120** is born by the substrate **S** and not by the pin holders **140** and **150**. In these embodiments, the contact pressure of the printing pins **120** are controlled by changing the weight of the printing pins **120**.

[0069] Referring to FIGS. 14A and 14B, pin holders **140a** and **150a** according to another embodiment of the invention are illustrated. The pin holders **140a** and **150a** are provided a means to vary the contact pressure of the printing pins **120** without changing the weight of the printing pins **120**. The pin holders **140a** and **150a** are provided with elastomeric member **160** to exert contact pressure for printing. The elastomeric member **160** may be a sheet-like membrane or a layer of foam positioned above the pins **20** and at a fixed distance **d** from the top surface of the planar members **141** and **152**. After the pins **20** make contact with the substrate S, the pin holders **140** and **150** are lowered further by the drop distance **h**. But because the elastomeric member **160** is in a fixed relation with the planar members **141** and **152** of the pin holders **140** and **150**, respectively, the elastomeric member **160** is lowered at the same time and presses against the head portions **26** of the printing pins **120**. By varying the distance **d** between the elastomeric member **160** and the planar members **141** and **152** and also the drop distance **h**, the contact pressure of the pins **120** exerted against the substrate **S** can be varied. For example, for a given configuration, where the distance d between the elastomeric member **160** and the planar members **141** and **152** are fixed, the contact pressure can be increased

by increasing the drop distance **h** because the head portions **26** of the printing pins **120** will compress further into the elastomeric member **160** causing the elastomeric member **160** to exert greater down force against the printing pins **120**. By judiciously selecting material and the physical parameters of the elastomeric member **160**, a wide range of contact pressures may be obtained. For example, in an embodiment where the elastomeric member **160** is a polymer foam, its overall thickness, foam cell size, cell density in the foam, the polymer material, and the foam backing, etc. may be varied. In an embodiment where the elastomeric member **160** is an elastomeric membrane, its overall thickness and elasticity of the membrane are some examples of the parameters that may be varied. Regardless of the particular elastomer used, it should not be compliant so that the printing pins recover immediately to its fully extended position in the pin holder when lifted off the substrate so that the pins are ready for the next printing cycle.

[0070] The microcontact printing pins described herein are especially useful for printing and manufacturing high quality microarrays of proteins, DNA, RNA, polypeptides, oligonucleotides and microarrays of other biological materials having spot volumes in the range of 10^{-10} picoliters to 100 nanoliters. The microcontact printhead device may also be used for printing and manufacturing high quality microarrays of other matters including, without limitation, solid semiconductor quantum dots or liquid dots containing various functional molecules, such as sensors, organic small molecules, organic polymers, solutions of organic polymers, dyes, inks, adhesives, molten metals, solders, glasses, and ceramic oxides.

[0071] While the foregoing invention has been described with reference to the above, various modifications and changes can be made without departing from the spirit of the invention. Accordingly, all such modifications and changes are considered to be within the scope of the appended claims.